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Optical technologies for antibacterial control of fresh meat on display

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ABSTRACT

Ozone and ultraviolet light are techniques used for microbiological control in foods that use different mechanisms of action to complement their antibacterial action. This study aimed to evaluate the complementarity of these antimicrobial techniques in the food safety of beef contaminated with *Escherichia coli*. The treatments of aqueous ozone and UV-C were evaluated in cycles, with each cycle having a dose of 69 mJ/cm^2 of light and 30 s of ozone spray at a concentration of 0.9 ppm, the time between each cycle was 1 h and repeated ten times. The 1.7 Log total of *E. coli* reductions corresponds to the colony sum of the amount reduced by treatments from the amount proliferated without treatment. The techniques were also evaluated in isolation, obtaining a significant reduction for UV-C Light and for aqueous ozone it maintained the microbial load controlling proliferation. The organoleptic properties of the meat were evaluated by checking the pH, quantification of proteins, and lipid oxidation. It was observed that the treatments did not cause significant changes in the meat samples, showing that the technologies have the potential to preserve food by avoiding an exponential proliferation of microorganisms without modification of their organoleptic properties.

1. Introduction

According to the World Health Organization (WHO), most foodborne illnesses are caused by microorganisms, including bacteria. The microbiological safety of food is established by the proliferation of deteriorating and pathogenic bacteria (Lorenzo et al., 2018), which will go beyond establishing consumer's safety, determining the shelf life of these foods. The technique chosen for the microbiological control in food (water vapor and high temperatures) can cause its deterioration, such as the degradation of proteins, changing its organoleptic properties (Guillén, Mir-Bel, Oria, & Salvador, 2017), changing its organoleptic properties, including the degradation of proteins (Dumay, Picart, Regnault, & Thiebaud, 2006). Also, microbial virulence mechanisms have adaptive responses to critical control temperature factors (Jeyaseelan, 2002). The use of acids and nitrites and nitrates modifying the properties of foods can also be harmful to human health (Gaudette & Pickering, 2013).

The severity of outbreaks of bacterial toxins in meat has been changing public awareness of the safety of its consumption, which is mainly caused by *Escherichia coli* (*E. coli*). These enteropathogenic bacteria are prominent causes of diarrhea that can cause systemic complications particularly irritable bowel (Hwang et al., 2014). The DNA of antibiotic-resistant bacteria from sick people has been found in agricultural environments; several bacteria carried by animals, often resistant to antimicrobials, can contaminate food from slaughter to processing (World Health Organization, 2017). If the pathogenic bacteria in food diseases become resistant to antibiotics, they can hardly be treatable, and more people can die from foodborne diseases.

UV light is an antimicrobial physical method that causes DNA damage by absorbing light by purines (200 and 300 nm) and pyrimidines (260 and 265 nm) by blocking bacterial replication. Repairs of DNA damage caused by UV are dependent on light dose and the bacterial environment. (Kowalski, 2009). The ozone is a chemical method, Food and Drug Administration (FDA) approved, that presents a critical feature of non-generation residues. The inactivation mechanisms of microorganisms by ozone can happen through the oxidation of proteins or fatty acids, which leads to the leakage of the microbial cellular content (Pandiselvam et al., 2019). Therefore, this research aims to evaluate the effect of aqueous ozone and UV-C light, combined or independent, to improve their use in foods to inactivate microorganisms and,

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consequently, preserve their characteristics, guaranteeing the food safety consumers of bovine meats.

2. Material and methods

2.1. Microorganism

Escherichia coli (American Type Culture Collection ATCC 25922 - United States) was used for contamination of meat samples. The pre-inoculum was performed with one ml of the microorganism suspended in 9 ml of Brain Heart Infusion (BHI) liquid culture medium at 37 °C, 150 rpm for 16 h. Five ml of this culture were inoculated in 45 ml of liquid BHI under the same temperature conditions as in a rotary type of incubator (model Q315IA Quimis® - Brazil). The bacteria were then centrifuged at 3000 rpm for 10 min (Centrifuge model 5702 Eppendorf® - Germany) and re-suspended in sterile phosphate buffer (phosphate-buffered saline, PBS: pH 7.2). Dilutions with PBS were made to adjust the inoculum to a concentration of 10^6 colony-forming units per milliliter (CFU/ml).

The homogenization technique was used for microbiological collection by collecting the sample with sterile forceps and inserted into a Falcon tube with 10 ml of diluent (self-sterile distilled water) and homogenized at Vortex tube shaker for 1 min. Serial dilutions were made and sown in Petri dishes, incubated in an oven at 37 °C for 24 h, to count the CFU/cm^2 (Gorman et al., 1997).

2.2. Meat

Beef samples (ruckled/red meat) were purchased from randomly selected butchers in the city of São Carlos, SP (Brazil). The samples were standardized for size with an area of 10 cm^2 and 2 mm thick. The meats were washed for 1 min with distilled water sob agitation before starting the experiments to remove the initial contamination. Then, the samples were filtered, and the distilled water was discarded. This procedure was repeated twice. The bacteria *E. coli* re-suspended in PBS were inoculated for 15 min in bovine meat.

2.3. Experimental design

Each test featured ten counter-hung samples located on top (simulating how meats are displayed for sale) of the prototype shown in Fig. 1 plus two more controls (initial and final). The experiments were performed on two different occasions for each condition UV-C, O_3, UV-C + O_3 and UV-C + O_3 alternating, with a total of 96 samples, 12 for each condition with 5 replicates (aliquots) for each cycle. The conditions of the samples between cycles are 2–4 °C and absence of light.

The effect of ozonated water and ultraviolet light on meat contaminated with microorganisms was evaluated independently and combined: 1. Experiments with UV-C light were performed in cycles, with a total of 10 irradiations, with the samples irradiated for 15 s (69 mJ/cm^2) with an interval of 1 h between them, totaling 10 h of the experiment; 2. Experiments with ozone in the aqueous phase (0.9 ppm) were performed in cycles, with a total of 10 sprays for 30 s with an interval of 1 h between them, totaling 10 h of the experiment; 3. Experiments with ozone in an aqueous phase and UV-C light combined in the same cycle, first applying 15 s of UV-C light and then 30 s of ozone spraying in an aqueous phase, totaling ten cycles and 10 h of the experiment; 4. Experiments with aqueous ozone and UV-C light were applied alternately in the cycles, totaling ten cycles and 10 h of the experiment.

2.4. Analysis of organoleptic qualities

The analyzes performed were carried out for cycles 1,3,6, and 10 of the combined treatment of UV-C light (15 s) and ozonated water (30 s of spraying) and a control sample. The choice was that since it had both treatments and changes, the treated samples would be the most affected due to increased exposure to antimicrobial agents for 4 samples to be considered in this treatment range, including mainly the lowest (cycle 1) and highest exposure (cycle 10) of meats.

2.4.1. Lipid oxidation

In the filtration method, the beef samples (5 g) were homogenized for 5 min with 25 ml of sterile distilled water. Twenty-five ml of 10% trichloroacetic acid were mixed with samples and left to react for 5 min, according to Siu and Draper (SIU & DRAPER, 1978). After this reaction, the samples were filtered with a qualitative paper filter with a diameter of 15 cm, 0.16 mm. Afterward, 5 ml of the filtrate was removed, and 1 ml of 0.06M 2-thiobarbituric acid (TBA) was added for 90 min at 80 °C and analyzed on the spectrophotometer Cary UV-VIS (Varian, Australia) at 532 nm wavelength. Compared to a "Zero" with the preparation of 2 ml of distilled water, 2 ml of trichloroacetic acid, and 1 ml of TBA. The results are shown as species reactive to TBA in mg of malonaldehyde MDA/kg of meat. For the calculations, the molar extinction coefficient of MDA was 1.56×105 M cm^{-1} with $n = 6$ per for each cycle.

2.4.2. Total proteins

Thermo Fisher's standard 100–1500 μg/ml microplate protocol for the Bradford Kit was followed. Five grams of the beef samples were

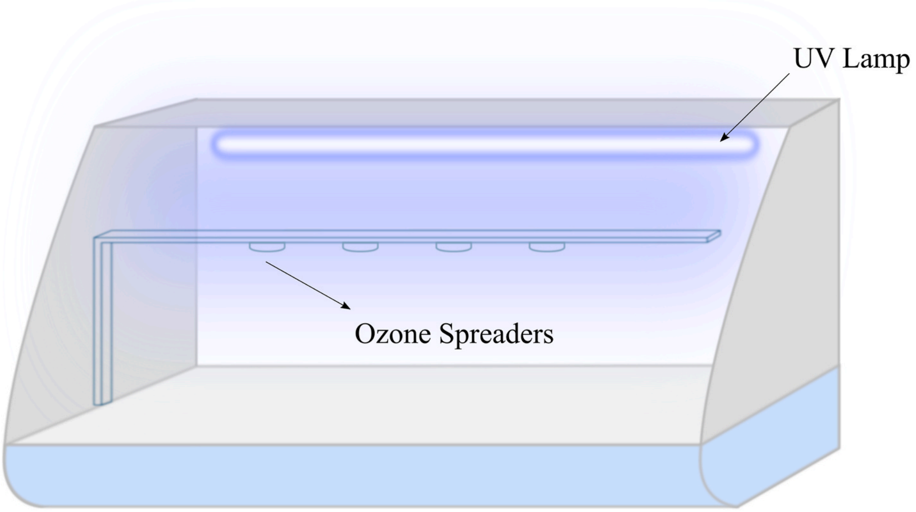

Fig. 1. Prototype design for food decontamination with UV-C light technology and ozone spreaders. By the author.

weighed and homogenized using an Ultraturrax shaker (model ultra380) to facilitate extraction. A 1: 1 protein extraction in distilled water was used for homogenization, which was always on cooling (ice application). The mixture was centrifuged at 10,000 rpm for 15 min at 4 °C, and the supernatant liquid was used for analysis with n = 6 per for each cycle.

2.4.3. pH

The bench pH meter used was Qualxtron® model QX1500. The sample (10 g) was ground with 90 ml of PBS solution in a blender for 1 min. The supernatant was collected, filtered, and measured. A pH measurement was made for each time or cycle, and each measurement was replicated twice (Hye-jin Kim, Sujiwo, Kim, & Jang, 2019).

2.4.4. Statistical analysis

According to their parametric distribution, for data with normal parametric distribution, the ANOVA One Way test was applied. The level of significance was 5% ($p < 0.05$). The results are presented by the mean and standard deviation (normal distribution).

3. Results

3.1. Light cycles

Each cycle of UV-C light was evaluated at every 1 h, with a delivered light dose of 69 mJ/cm^2. The results of applying multiple doses over time are shown in Fig. 2.

The results showed a reduction of *E. coli* 1.27 Log (CFU/cm^2) in the last cycle (10) compared to the control sample (0), and a bacterial growth rate was observed with 0.91 Log CFU/cm^2 more in the control (0 *) in comparison with the initial control (0). Proving that without any type of treatment there will always be bacterial growth.

No significant reductions in cycles were observed when comparing the treated groups with the initial control group (0) due to the low dose of light applied which was sufficient to avoid the exponential proliferation of the microorganism in the meat.

The authors deduced that the total bacterial reduction of 2.17 obtained is the sum of the Log of dead microorganisms and the growth of the final control sample (0 *).

3.2. Ozone cycles

Ten cycles of aqueous ozone were carried out at a concentration of 0.9 ppm sprayed on the meat, and a significant microbial reduction ($p < 0.05$) was not observed concerning the initial control sample (Fig. 3). However, there was a significant growth among the untreated samples at the end of the experiment (final control sample), which was compared with all cycles, obtaining a growth of 0.6 Log (CFU/cm^2) for that a total reduction in this treatment was 0.6 Log of *E.coli* that was the value of proliferation that this technique was able to prevent.

3.3. Combined cycles of UV-C light and aqueous ozone

The evaluation of UV-C light and aqueous ozone in beef is shown in Fig. 4. It can be seen that, in almost all cycles of the graph, the samples treated with the combination were significantly different ($p < 0.05$) of untreated samples. The final control samples collected at the end of the experiment showed more significant microbial growth than the results of all cycles and initial controls, confirming that the meat not treated with technologies for decontamination tends to increase bacterial proliferation, even maintaining temperatures not ideal for growth.

According to Fig. 4A, significant reductions were observed in all treated cycles. In cycles 5 and 8, there was a reduction of *E. coli* of 0.7 Log (UFC/cm^2). The initial microbial load and without proliferation (6.6 Log CFU/cm^2) were maintained in the other cycles. The final control sample (0 *) showed microbial growth of 1 Log (CFU/cm^2) approximately about the treated samples. In order to evaluate the technologies alternately (Fig. 4B). The results showed a significant reduction in microbial load for cycles 2–10 compared to the initial control sample, with these reductions around 0.7 Log (CFU/cm^2), thus avoiding the

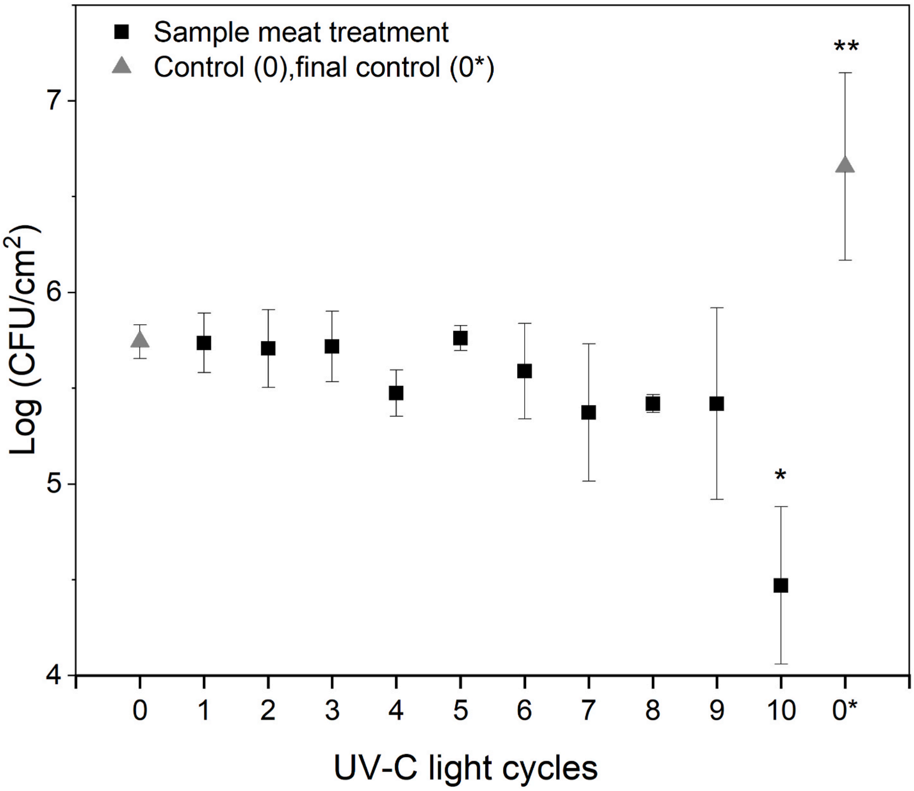

Fig. 2. Irradiation of UV-C light in beef samples contaminated with *E. coli* during 1-h cycles. The light dose for each cycle was 69 mJ/cm^2. In the graph, (■) represents the meat samples that were treated, and (▲) represents the meat samples that did not receive irradiation, being control samples evaluated at the beginning (0) and at the end of the experiments (0*). Values express the mean and standard deviation. * Significantly different from the control ($p < 0.05$). ** Significantly different from the samples ($p < 0.05$). By the author.

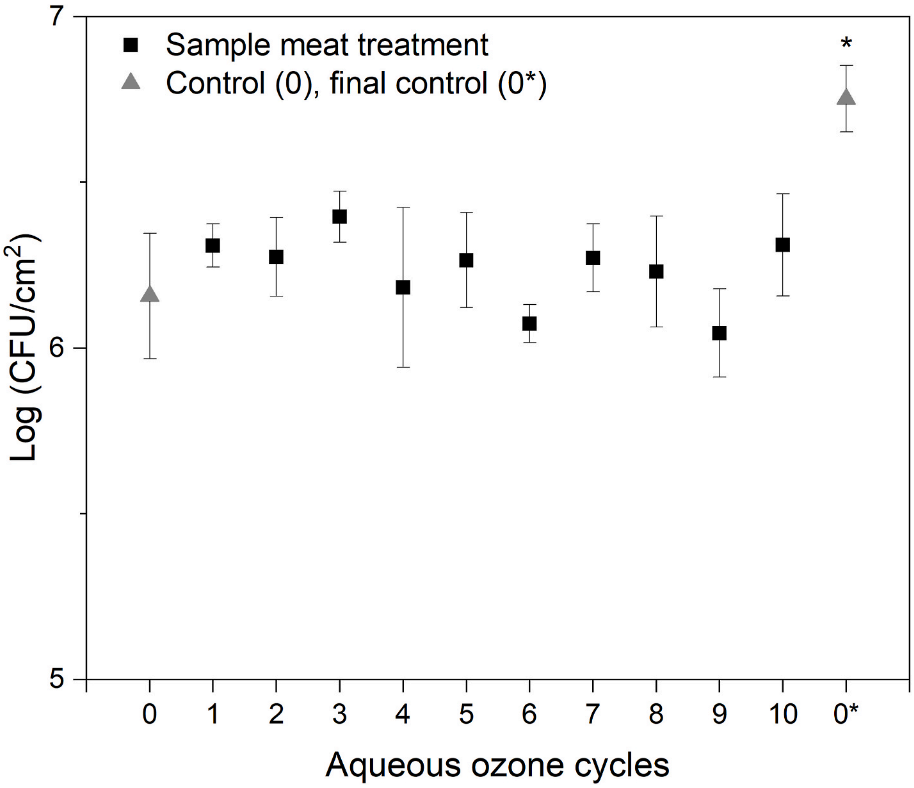

Fig. 3. Aqueous ozone sparging cycles (0.9 ppm) in beef samples contaminated with *E. coli* during 1-h cycles. In the graph, (■) represents the meat samples that were treated, and (▲) represents the meat samples that did not receive sparging of aqueous ozone, being control samples evaluated at the beginning (0) and at the end of the experiments (0*). Values express the mean and standard deviation. * Significantly different from the control ($p < 0.05$). By the author.

proliferation of microorganisms. In both treatments, a total reduction is 1.7 Log of *E. coli* approximately because the reduction in treated samples was 0.7 Log for almost cycles and the proliferation in untreated samples was 1 Log.

3.4. Organoleptic qualities

Table 1 shows the results of the tests for pH, protein quantification, and lipid oxidation for bovine meat treated in combination with ultraviolet light (UV–C) and aqueous ozone.

The pH measurement was performed for a control sample and treated samples. The controls sample has a normal pH, and the treated samples did not have a significant difference in pH change. Thus, this result shows that the action of the combined treatments on the meat does not affect the pH; therefore, it does not change the meat's quality.

The acceptance threshold for beef and meat products of 1 mg MDA/kg of meat was found (Gray, Gomaa, & Buckley, 1996). Table 1 shows the values acquired for evaluating the TBA test after combined treatments with UV-C light and aqueous ozone in beef, observing that there was no significant difference for the treated samples compared to the control and between each cycle. Considering that the maximum acceptable TBA value is 1 mg MDA/kg of meat, and those obtained were lower, inferring those combined treatments did not increase the malonaldehyde content.

The Bradford protein assay was used to measure the total protein concentration of the meat samples that can indicate the total protein content correlated with the treatments, indicating the quality of the meat. A standard curve was constructed with bovine serum albumin (BSA) serial dilutions to obtain sample concentrations (Thermo Fisher Bradford kit) of Table 1. The calculations were made with the line equation, $Y = 0.4247 + 0.66816 * x$ with a of $R^2 = 0.99$.

The cycles showed no significant difference with the control in the number of total proteins in the samples. On the other hand, between cycles, there was a difference between cycle 6 and cycle 9, with the highest total proteins in the last cycle. A strong hypothesis is that the samples are unique and heterogeneous, and the total proteins can vary. Applications with treatments do not decrease the total amount of protein, so technologies do not damage this parameter.

3.5. Microbiological analysis

Bacteria are observed on the surface of food, more specifically in the meat, that does not undergo microbiological control processes will always tend to bacterial proliferation, even with controlled temperature. In the present study, UV-C and aqueous ozone technologies were used to inactivate microorganisms that resulted in significant reductions in logarithmic scale. It was also verified that the contaminated samples (without any treatment) showed an increased in bacterial cells. For UV-C experiments using in meets, similar results were found for ground meat contaminated with four strains of *Salmonella*. They showed a 1.15 Log (CFU/g) reduction, using UV-C light for 30 s (Yeh, de Moura, Van Den Broek, & de Mello, 2018). Another study performed with contaminated beef (Korean Hanu cattle breed) by mesophilic bacteria showed no statistical difference in bacterial inactivation between treated and control samples; however, it showed that the irradiated samples preserved the same amount of microbial load throughout storage days (Hyun-jung Kim, Lee, & Eun, 2014). Recent studies evaluating that UV-C (254 nm) in beef, chicken, and pork contaminated with E.coli ATCC 25922 irradiated with lamps for 1, 2, 3, 4, 5, and 10 min showed reductions in the number of *E. coli* in beef around (1.0 ± 0.2) Log (CFU/ml) after 5 min of exposure, in chicken and pork, reductions of (1.6 ± 0.7) Log (CFU/ml) and (1.6 ± 0.4) Log (CFU/ml) after 4 and 10 min of irradiation, respectively (Corrêa et al., 2020). These studies corroborate the results obtained in the present study.

The microbiological reductions using aqueous ozone were similar to those presented by studies reported in the literature. The study by

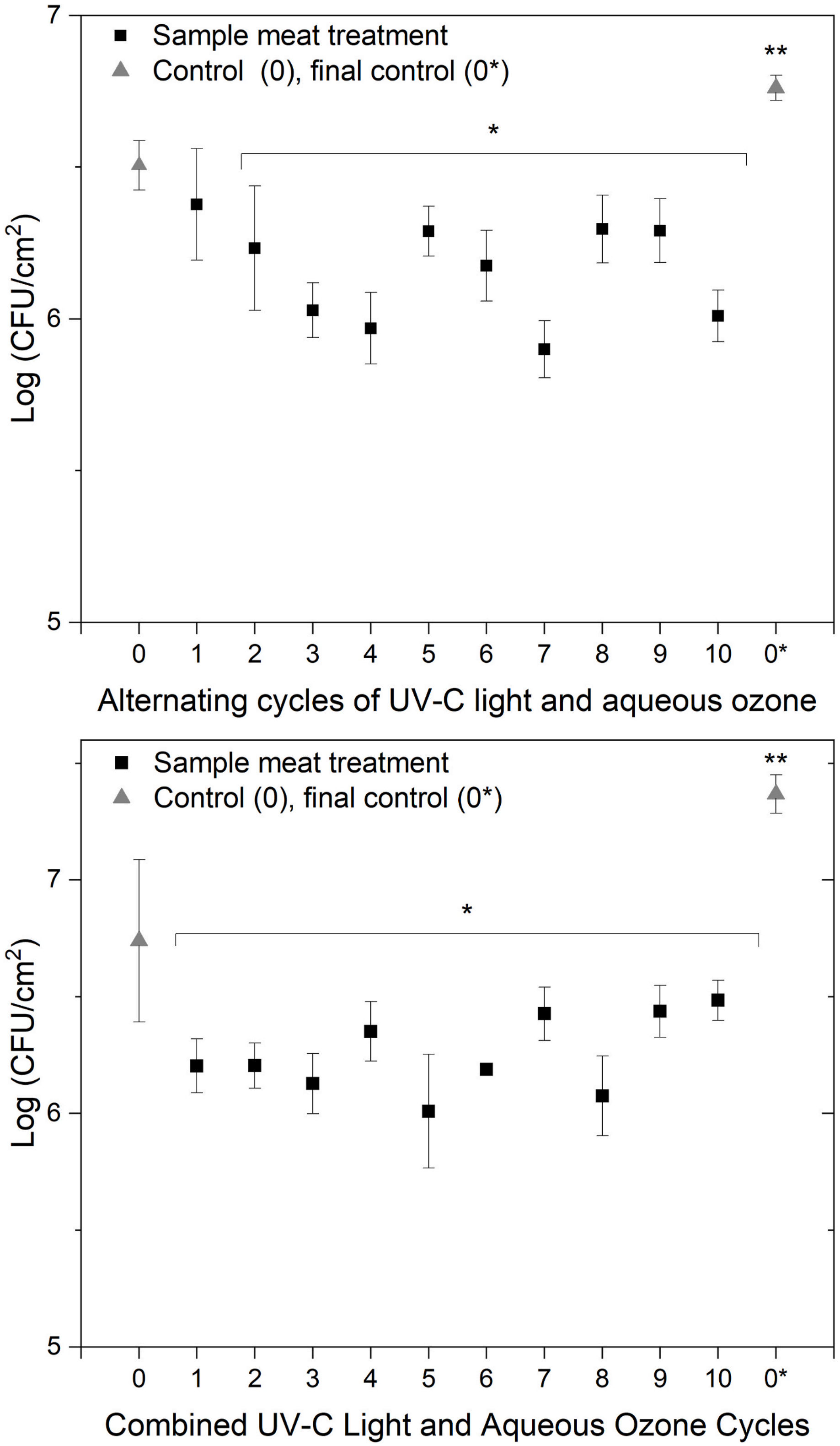

Fig. 4. Combination and alternating of aqueous ozone with UV-C light. (A) Combined simultaneous cycles of UV light and aqueous ozone, (B) Alternating cycles of UV-C light and aqueous ozone in beef samples contaminated with E.coli. In the graphs, (■) represents the meat samples that were treated, and (▲) represents the meat samples that did not receive treatments, being control samples evaluated at the beginning (0) and the end of the experiments (0*). Values express the mean and standard deviation. * Significantly different ($p < 0.05$), ** Significantly different from all ($p < 0.05$). By the author.

Table 1
Measures of lipid oxidation (TBA), protein quantification and pH, in beef treated with aqueous ozone and UV-C light. In the table shows control sample and 1,3,6 and 10 cycle. Values are presented with the mean and standard deviation.

Cycle sample	Lipid oxidation (mg/kg)	Total Protein (mg/g)	pH
0	0.12 ± 0.11	9.17 ± 0.68	5.76 ± 0.07
1	0.12 ± 0.04	8.80 ± 0.51	5.88 ± 0.15
3	0.08 ± 0.04	8.75 ± 0.65	5.90 ± 0.14
6	0.07 ± 0.00	8.41 ± 0.99	5.88 ± 0.14
10	0.12 ± 0.06	9.60 ± 0.59	5.88 ± 0.15

Castillo et al. consisted of evaluating the synergism of washing with warm water (28 °C) and aqueous ozone (95 ppm) in bovine carcasses contaminated with *E. coli* O157: H7 and *Salmonella typhimurium* (Castillo, McKenzie, Lucia, & Acuffi, 2003). The reductions obtained with ozone treatment were not significant compared with the control samples' tests and those obtained only with washing with water. Ozone synergism was evaluated at temperatures from 45 °C to 75 °C, using meat contaminated with *Clostridium perfrigens*. The results showed that the combination obtained a decrease of 1.5 Log (CFU/g) in vegetative cells using 5 ppm of aqueous ozone for 5 min, followed by treatment at 45 °C for 30 min and a reduction of 2.09 Log (UFC/g) using 5 ppm, followed by treatment at 55 °C. However, in these studies, the samples analyzed individually with ozone and at 45 °C, and 55 °C did not show any significant difference in microbial inactivation concerning the control sample. In this investigation, cell death for temperatures above 65 °C has also been reported; however, the meat appearance has changed, which has been deteriorated and has a cooked appearance (darkening of the meat surface) (Novak & Yuan, 2004).

In another study, Atlantic salmon fillets were inoculated with *Listeria innocua*, which evaluated the influence of aqueous ozone spray treatments at concentrations of 1 mg/L and 1.5 mg/L of ozone, obtaining for 1 mg/L with 3 applications of 1.17 Log (CFU/g) of microbial inactivation. Aerobic bacterial populations were significantly reduced by 1.5 mg/L ozone sprays on day 0 compared to controls. However, in contrast to the effectiveness of treatments, there were no significant reductions from multiple sprays to 1.5 mg/L concentration. Although the results show significant differences, they did not specify the spray contact times with the samples, which is an significant parameter for reductions and final food quality (Crowe, Skonberg, Bushway, & Baxter, 2012).

Another study reported an aqueous ozone study at a concentration of 12 ppm with spray interventions on fresh beef, with spraying times of 90 s every 30 min for 12 h. The cooling by aqueous ozone spray reduced the aerobic bacteria by 0.99 Log, and for *E. coli* O157: H7, the reduction was 1.46 Log (Kalchayanand, Worlie, & Wheeler, 2019), showing the effectiveness of the technology.

Although the microbial reductions were smaller in the present study, they may be related to exposure of the samples to ozone, the concentration of aqueous ozone, and the long waiting time between each cycle. Therefore, it has been shown that ozone in water, made available in sequential sprinkling mode, helps preserve the initial microbial load, possibly increasing shelf life and food quality.

In this study, the combination of UV-C and ozone was studied to improve the antimicrobial action and try to reach cell death targets in different ways, as the microorganisms approach the stationary phase of growth to secrete proteases that degrade the connective tissue between the muscle fibers of the meat, allowing bacteria to penetrate the meat.

The alternating cycles of UV-C and ozone were shown in meat samples contaminated with *E. coli*, there is a constant reduction behavior until the fourth cycle, which hypothesizes that the effect of the associated antimicrobial action is greater than the isolated use of ozone and UV-C, considering that the bacterium was still in its spontaneous growth phase and was probably still on the surface of the meat. Although in some remaining cycles there were increases in the microbial load, all of them keep loads lower than the initial control sample.

Finally, we understand that two antimicrobial techniques used together are less efficient against microorganisms than when used alone. Treatment with UV-C light obtained a reduction of 1.27 Log in the last cycle and 0.6 Log for aqueous ozone, similar to that of the combined cycles of UV-C light and 0.7 Log of aqueous ozone (CFU/cm^2). One hypothesis suggested that photons of light are scattered in ozonated water on meat. Therefore, this scattering of light through the water can interfere with the transport of photons to the surface of the meat where the bacteria are found during its logarithmic phase of growth, but the use of ultraviolet light in isolation is light dose-dependent and can even cause visual damage to oxidative damage (dry appearance) after processing the meat, unlike the aqueous ozone technique that helped to maintain the hydration of the meat samples (Davis, 1955; Gill & Penney, 1977; Sommers, Scullen, & Sheen, 2016).

3.6. *Organoleptic quality*

In the food industry, microbiological control is of paramount importance to ensure food safety. Depending on the technique chosen to contain these microorganisms, food degradation can occur. For example, parameters such as pH are essential in the quality of beef. A change in pH can change tenderness, texture, color, ability to retain water and alter bacterial growth (Ruiz-Hernández, Sosa-Morales, Cerón-García, & Gómez-Salazar, 2021). Based on the literature, pH values determine the meat's quality after slaughter; when the animal dies, there is a pH decay up to 24 h where it stabilizes at values of 5.5–5.8. In the evaluation carried out for meats treated with ultraviolet light and aqueous ozone in a combined manner, there was no change in pH.

Lipid oxidation is considered a complex process where reactive oxygen species react with the polyunsaturated fatty acids present in meat, causing their deterioration, defining the food's sensory quality and shelf life (Amaral, Viana, & Caetano, 2018). The acceptance threshold for meat and meat products of 1 mg MDA/kg of meat was found (Gray et al., 1996). However, these results made with ultraviolet light and aqueous ozone treatments fit the threshold of acceptance.

4. Conclusions

This study showed that it is possible to insert new microbiological control parameters for the routine exposure situations of fresh meat sales subject to contamination in the display equipment. The disinfection method used is suitable for other samples of meat and even other foods, considering that bacteria are on its surface when it is proliferating. The ideal conditions would be 12 cycles using UV and ozone combined to reduce surface proliferation of *E. coli* maintaining the organoleptic properties of meat, which is a food spoilage problem.

CRediT authorship contribution statement

Shirly Lara Perez: Writing – original draft, Investigation, Formal analysis, Methodology, Validation, Conceptualization. **Daniel Jose Chianfrone:** Data curation, Resources, Software. **Vanderlei Salvador Bagnato:** Project administration, Funding acquisition, Conceptualization. **Kate Cristina Blanco:** Supervision, Visualization, Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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